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Anti-*Helicobacter pylori* Activity of *Bifidobacterium* spp.

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Abstract The inhibitory effects of different *Bifidobacterium* spp. on the growth of *Helicobacter pylori* (HP) were investigated. A significant suppression of HP growth occurred only when HP was inoculated onto a petri dish containing 0.1 mg/ml of *Bifidobacterium* spp. When HP was separately cultured with *B. breve* K-110, *B. catenulatum* K-309, *B. magnum* K-311, *B. magnum* K-321, and *B. cuniculi* K-513, the urease activity was also inhibited by these *Bifidobacterium* spp. Therefore, it appears that these *Bifidobacterium spp.* excrete a heat-labile inhibitory component for HP growth into the culture medium. Although most organic acids produced by the *Bifidobacterium* spp. inhibited the growth of HP, the HP growth was not inhibited by the physiological concentrations of organic acids produced in bifidobacteria-cultured media. Accordingly, these results suggest that some *Bifidobacterium* spp. may produce antibiotic-like compounds (bacteriocins).

Key words: *Helicobacter pylori*, *Bifidobacterium* spp., bacteriocin

Helicobacter pylori (HP) was isolated from the gastric antrum of chronic gastritis patients by Warren and Marshall in 1983 [1]. HP produces a vacuolating toxin and its toxicity may be potentiated by urease-mediated ammonia production [2]. Since HP urease is considered to play a critical role in the pathogenesis of gastritis and peptic ulcers, the eradication of this bacteria and inhibition of HP urease are important for the treatment of patients with gastroduodenal diseases [3, 4].

Lactic acid bacteria have been considered as the most beneficial probiotic organisms contributing to inhibition of harmful and putrefactive intestinal bacteria. An improvement of lactose malabsorption in humans, the enforcement of immune functions, and the prevention of cancer are important [5]. Among them, *Bifidobacterium* spp. has been considered as one of the most beneficial probiotic organisms that can improve the health of humans, since it is one of the major bacterial flora in human intestine and exhibits various kinds of biological activities. However, the anti-HP activity of lactic acid bacteria, especially *Bifidobacterium* spp., has not been studied, except that Bhatia *et al.* [6] and Lee *et al.* [7] reported *Lactobacillus acidophilus* inhibiting the growth of *H. pylori in vitro*. Accordingly, an investigation of the anti-*H. pylori* activity of *Bifidobacterium* spp. should be valuable to determine the inhibitory effects of *Bifidobacterium* spp. against the growth of HP *in vitro*. Consequently, these *Bifidobacterium* spp. inhibiting harmful enzymes of human intestinal microflora and three bifidobacteria, *B. breve* K-110, *B. breve* K-111, and *B. infantis* K-525, were isolated from healthy Korean intestinal microflora. These strains exhibited strong *in vitro* inhibitory activities against the harmful enzymes of human intestinal microflora [8, 9].

Culture of Lactic Acid Bacteria

B. breve K-110, *B. breve* K-111, and *B. infantis* K-525, which were isolated from fecal samples of healthy Korean subjects as lactic acid bacteria with potent biological activities, were separately inoculated into a general anaerobic medium (Nissui Pharm. Co. Ltd., Japan). Then, each cultured bacteria was inoculated into 5 l of tryptic soy broth containing 0.01% sodium thioglycolate and 0.1% ascorbic acid. The cultured bacteria were centrifuged at 4,500 ×g for 20 min. The precipitates were washed with saline and then used as samples. The samples were suspended in 10 mM phosphate buffer, sonicated (Ultra Heat system, U.S.A.), and centrifuged. The supernatants (cytosolic fraction) and precipitates (cell wall fraction) were also used as the samples.

Assay of HP Growth-Inhibitory Activity

HP growth-inhibitory activity was measured according to our previous method [10]. One milliliter of each sample of *Bifidobacteria* or each compound was placed in a petri dish containing 7 ml of an unsolidified brucella agar supplemented with 7% horse serum. The final concentration of each *Bifidobacterium* spp. was 1, 0.1 and 0.01 mg/ml, and the final concentration of each organic acid was 16, 8, 4, 2, 1,

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Table 1. Inhibitory effect of *Bifidobacterium spp.* on the growth of *H. pylori.*

Microbe	Inhibition					
	Intact cell		Cytosolic Fr.	Cell wall Fr.	Supernatant[b]	
	Untreated	Heated			Untreated	Heated
B. cholerium K-103	+++[a]	-	-	-	-	-
B. bifidum K-105	+++	-	-	-	-	-
B. breve K-110	+++	-	-	-	+	-
B. breve K-111	+++	-	-	-	-	-
B. catenulatum K-309	+++	-	-	-	+	-
B. magnum K-311	+++	-	-	-	+	-
B. magnum K-321	+++	-	-	-	+	-
B. minimum K-506	+++	-	-	-	-	-
B. cuniculi K-513	+++	-	-	-	+	-
B. infantis K-525	+++	-	-	-	-	-

[a]Growth-inhibitory potency was measured at 10 μg/ml of *Bifidobacterium* spp.: +++, strong inhibition; ++, moderate inhibition; +, weak inhibition; -, no inhibition.
[b]Heated at 100°C for 10 min.

0.5, 0.25, and 0.125 mg/ml. An approximately 5×10^5 colony-forming units (CFU) of HP was inoculated onto the agar plates and cultured microaerobically for 3 days at 37°C in an anaerobic jar (85% N_2, 10% CO_2, 5% O_2). The minimum inhibitory concentration (MIC) range was determined after an incubation period of 72 h. Ampicillin was used as a positive control. All experiments were conducted in triplicates.

Effect of *Bifidobacterium* spp. on Growth of *H. pylori*

The inhibitory effects of each *Bifidobacterium* spp. on the growth of HP are shown in Table 1. All the *Bifidobacterium* spp. tested strongly inhibited the growth of HP at 0.1 mg/ml. This inhibition only occurred when HP was inoculated onto a petri dish containing intact fresh *Bifidobacterium* spp. However, when the *Bifidobacterium* spp. were treated in boiling water for 5 min, the growth of HP was not inhibited at more than 1 mg/ml. Furthermore, the cytosol and cell wall fractions of each *Bifidobacterium* spp. did not inhibit the growth of HP. Each *Bifidobacterium* spp. was cultured in TS broth and then centrifuged. The supernatant was sterilized with a 0.22 μm millipore and the anti-*H. pylori* activity was measured. The sterilized supernatants of *B. breve* K-110, *B. catenulatum* K-309, *B. magnum* K-311, *B. magnum* K-321, and *B. cuniculi* K-513, all inhibited the growth of HP. However, they did not all lose the inhibitory activity after being treated with boiling water for 5 min. The inhibitory activity of the organic acids produced by human intestinal bacteria as well as *Bifidobacterium* spp. on the growth of HP was investigated (Table 2). Most organic acids inhibited the growth of HP. Acetic acid exhibited the strongest inhibitory activity. However, the minimal inhibitory concentration (MIC) of these organic acids against HP was higher than the concentration of organic acid produced in the bifidobacteria-cultured media. In addition, when these organic acids were adjusted to pH 7.2 with NaOH, they did not exhibit the same inhibitory activity. Furthermore, the inhibitory effect of these *Bifidobacteria* for HP urease was investigated, when HP were cultured with these *Bifidobacteria* (Table 3). *B. breve* K-110, *B. catenulatum* K-309, *B. magnum* K-311, *B. magnum* K-321, and *B. cuniculi* K-513 also inhibited HP urease activity as well as HP growth. These results suggest that some *Bifidobacteria* may produce antibiotic-like compounds (bacteriocins), which are heat-labile.

Table 2. Minimum inhibitory concentration (MIC) of organic acids produced by human intestinal microflora on the growth of *H. pylori.*

Compound	MIC (mg/ml)					
	HP ATCC43504	HP NCTC11637	HP NCTC11638	HP 82516	HP 82548	HP 4
Formic acid	1	2	1	2	2	1
Acetic acid	0.5	1	1	2	1	0.5
Propionic acid	2	4	2	4	4	2
Butyric acid	2	4	2	4	4	4
Lactic acid	2	2	2	4	4	2
Ampicillin	0.001	0.001	0.001	0.001	0.002	0.002

Table 3. Inhibitory effect of *Bifidobacterium* spp. on the final pH of the medium and HP urease activity.

Bifidobacterium spp.	Final pH	Urease Inhibition (%)
B. cholerium K-103	5.6	31
B. bifidum K-105	5.6	21
B. breve K-110	5.5	68
B. breve K-111	5.5	24
B. catenulatum K-309	5.5	69
B. magnum K-311	5.4	76
B. magnum K-321	5.4	74
B. minimum K-506	5.4	9
B. cuniculi K-513	5.4	70
B. infantis K-525	5.5	0

Compared to the antibiotics in clinics, the isolated *Bifidobacterium* spp. showed relatively weak inhibitory effects on the growth of HP. However, resistant pathogens to current antibiotics and their side effects have appeared [9, 10]. Accordingly, dairy products containing lactic acid bacteria, even if they are not potent growth inhibitors, may be able to contribute in some degree to prevent gastritis.

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References

1. Warren, J. R. and B. J. Marshall. 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* **1:** 1273–1275.
2. Tsujii, M., N. Kawano, S. Tsujii, H. Fusamoto, T. Kamada, and N. Sato. 1992. Mechanism of gastric mucosal damage induced by ammonia. *Gastroenterology* **102:** 1881–1888.
3. Eaton, K. A., C. L. Brooks, D. R. Morgan, and S. Krakowka. 1991. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infec. Immun.* **59:** 2470–2475.
4. Ki, M.-R., S.-K. Yun, W.-J. Lim, B.-S. Hong, and S.-Y. Hwang. 1999. Synergistic inhibition of membrane ATPase and cell growth of *Helicobacter pylori* by ATPase inhibitors. *J. Microbiol. Biotechnol.* **9:** 414–421.
5. Salminen, S., M. Deghton, and S. Gorbach. 1974. Lactic acid bacteria in health and disease, pp. 199–226. *In* S. Salminen and A. von Wright (eds.). *Lactic Acid Bacteria*, Marcel Dekker, Inc., New York, U.S.A.
6. Bhatia, S. J., N. Kochar, P. Abraham, N. G. Nair, and A. P. Mehta. 1989. *Lactobacillus acidophilus* inhibits growth of *Campylobacter pylori*. *J. Clin. Microbiol.* **27:** 2328–2330.
7. Lee, Y., E. Shin, J. Lee, and J. H. Park. 1999. *Lactobacillus acidophilus* inhibits the *Helicobacter pylori* adherence. *J. Microbiol. Biotechnol.* **9:** 794–797.
8. Park, H.-Y., E.-A. Bae, M. J. Han, E.-C. Choi, and D.-H. Kim. 1998. Inhibitory effect of *Bifidobacterium* spp. isolated from a healthy Korean on harmful enzymes of human intestinal microflora. *Arch. Pharm. Res.* **21:** 54–61.
9. Han, M. J., H.-Y. Park, and D.-H. Kim. 1999. Protective effect of *Bifidobacterium* spp. on experimental colon carcinogenesis with 1,2-dimethylhydrazine. *J. Microbiol. Biotechnol.* **9:** 368–370.
10. Bae, E.-A., M. J. Han, N.-J. Kim, and D.-H. Kim. 1998. Anti-*Helicobacter pylori* activity of herbal medicines. *Biol. Pharm. Bull.* **21:** 99–992.